Blood Glucose levels and Dyslipidemia in Chronic Periodontitis Patients: A Case Control study

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CERTIFICATE

This is to certify that **Dr.S.M.BAGGIALAXMI**, Post graduate student (2007-2010) in the Department of Periodontology, Tamilnadu Government Dental College and Hospital, Chennai- 600 003 has done this dissertation titled **"Blood Glucose levels and Dyslipidemia in Chronic Periodontitis Patients: A Case Control study "** under our direct guidance and supervision in partial fulfillment of the regulations laid down by The Tamilnadu Dr M.G.R. Medical University, Chennai- 600 032 for **M.D.S., (Branch –II) PERIODONTOLOGY DEGREE EXAMINATION.**

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ABSTRACT

BACKGROUND

Several studies have indicated that subjects with periodontal disease may have a higher risk for cardiovascular disease when compared to subjects with a healthy periodontium *(Mattila et al 1989, Beck et al 1998, Loesche 1998*).
Also, Periodontitis & Cardiovascular disease may share common risk factors such as Smoking, Diabetes, Ageing, and Male genders.

AIM

To measure the level of fasting plasma lipid profile and blood glucose levels in chronic periodontitis patients and compare and correlate the results with those obtained in age and sex matched healthy individuals.

MATERIALS AND METHODS

30 subjects with chronic periodontitis divided into mild, moderate and severe study group based on CAL and 30 age and sex matched healthy controls participated in this study. Venous blood sample was collected by experienced trained person in the morning after an overnight fast and analyzed for serum triglycerides, total cholesterol LDL, HDL and blood glucose in the study and control group. Periodontal parameters, namely, probing pocket depth and CAL were recorded on full mouth basis at 6 sites per tooth. All measurements were made using William's periodontal probe in both study and control group.

RESULT

Triglycerides were significantly higher in periodontally diseased subjects ($P<0.000$) when compared with controls. When the mild, moderate and severe periodontitis patients in the study group were clumped as one group and compared with controls, statistically significant values were obtained for Total Cholesterol ($P<0.002$)and Triglycerides ($P<0.000$). Also, we found significantly higher values in blood glucose levels in patients than in the control group.

CONCLUSION

It is likely that severity and poor control of periodontal disease might affect the level of lipids and glucose in blood which may increase the risk for CVD. Therefore, when periodontitis is newly diagnosed in patients with atherosclerotic CVD, periodontists and physicians managing patients with CVD should closely collaborate to optimize CVD risk reduction & periodontal care

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ABBREVIATIONS USED

AAP American Academy of Periodontology

APR Acute Phase Response

CAD Coronary Artery Disease

CAL Clinical Attachment Level

CEJ Cemento Enamel Junction

CHD Coronary Heart Disease

CVD Cardio Vascular Disease

EDTA Ethylene Diamine Tetra acetic acid

GCF Gingival Crevicular Fluid

GK glycerol kinase

GOD glucose oxidase

GPO glycerol-3-phosphate oxidase

HDL High Density Lipoprotein Cholesterol

IDDM Insulin Dependent Diabetes Mellitus

IHD Ischemic Heart Disease

IL InterLeukin

LDL Low Density Lipoprotein Cholesterol

LPL Lipoprotein Lipase

LPS Lipopolysaccharide

MI Myocardial Infarction

PMN PolyMorphoNuclear Leukocytes

PPD Probing Pocket Depth

SMC Smooth Muscle Cells

TC Total Plasma Cholesterol

TGL Triglycerides

TNF Tumor Necrosis Factor

INTRODUCTION

Periodontal diseases are among the most prevalent and widely spread diseases worldwide contributing mainly to tooth morbidity and mortality. It is the chronic immuno-inflammatory response to the bacteria and their products may result in periodontal destruction.

Periodontal disease may be more frequent and severe in individual with long duration of the disease and those with systemic complication. On the other hand, it may also occur in patients with well controlled systemic conditions. There is increasing evidence that periodontal infections may play a role in systemic disease not only in ill and immuno compromised individuals, but also in healthy individuals.

Infection produces changes in the lipid metabolism that may favor atherosclerosis. The initiation of atherosclerosis is due to focal accumulation of lipids. Periodontitis and Atherosclerosis have complex etiologies, genetic and gender predispositions and potentially share many risk factors.

Diabetes mellitus, caused by the malfunction of insulin-dependent glucose and lipid metabolism, presents with the classical triad of symptoms: polydypsia, polyuria, and polyphagia which are often accompanied by chronic fatigue and loss of weight. Complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, and cardiovascular disease.. Periodontitis being considered as the sixth complication of diabetes.

Periodontitis is caused by a small group of gram negative bacteria present on the root surfaces as biofilms. Lipopolysaccharide (LPS) and other microbial substances gain access to the gingival tissues, initiate and perpetuate immunoinflammation, resulting in the production of high levels of proinflammatory

cytokines. These induce production of matrix metalloproteinases which destroy the connective tissues of the gingiva, cementum and periodontal ligament, and prostaglandins which mediate alveolar bone destruction. Periodontitis may enhance susceptibility to systemic diseases in several ways. LPS and proinflammatory cytokines from inflamed periodontal tissue may enter the circulation in pathogenic quantities. This will result in increase in serum lipid levels and state of insulin resistance. Many studies suggest that chronic low level systemic exposure to LPS leads to generalized alteration in lipid and glucose metabolism. Thus periodontitis induced insulin resistance & hyperlipidemia if longstanding or chronic are considered to be precursors to active diabetes and cardiovascular diseases due to sustained elevations of proinflammatory cytokines.

In addition, Periodontitis and certain systemic diseases, such as cardiovascular disease share common risk factors including smoking, male gender, race/ethnicity, stress, genetic and aging. There is an abundance of evidence that chronic periodontitis, especially severe periodontitis in early life, significantly enhances risk for systemic disease. The purpose of this study is to demonstrate how untreated periodontitis influence the general health by altering the lipid and glucose metabolism.

AIM AND OBJECTIVES

The aim of the present study was to determine whether there is an alteration in the levels of blood glucose and lipid profile in patients with Chronic Periodontitis, when compared to individuals with healthy periodontium.

For this purpose, the following objectives were undertaken:

1. To compare the fasting blood glucose and lipid profile levels in individuals with healthy periodontium (Control Group) and Chronic Periodontitis (Study Group).
2. To correlate the levels of blood glucose and lipid profile of the Study Group with the clinical parameter of Clinical Attachment Loss within the same group.
3. Whether the alteration in the blood biochemical parameter increases with increase in the severity of the disease?

REVIEW OF LITERATURE

HYPERLIPIDEMIA AND CARDIOVASCULAR DISEASE

For more than half century, observational epidemiological studies have investigated whether elevated plasma lipids has any association with an increased risk of coronary heart disease.

Oral sepsis was first introduced into the medical literature in a report entitled Oral sepsis as a cause of systemic disease by **William Hunter** in **1900**[99]. This was then superseded by focal infection, introduced by **Frank Billings** in **1921**[9].

Focal infection has been continued to be explored as a possible cause or exacerbating factor of some systemic conditions, but presently it is being evaluated on scientific basis. The concept of focal infection, while shifting in and out of favor as a pathogenic mechanism has always been recognized as being potentially causal in bacterial endocarditis due to bacteremia.

Albink et al[3] was the first to report an association between triglyceride and myocardial infarction (MI) in a case-control study published in **1959**. Since that time, many case control or cross-sectional design have been reported with an increased risk of coronary heart disease (CHD).In many of the studies, the association of triglyceride and outcome was analyzed, after controlling for either total plasma cholesterol (TC) or low density lipoprotein cholesterol (LDL-C). In each study, the association of triglyceride with CHD persisted after taking into account TC or LDL-C level. Some of the studies in which possible confounding effects of high density lipoprotein

cholesterol (HDL-C) were also considered, the triglyceride association remained significant.

Elevated concentrations of serum cholesterol and triglycerides have both been implicated in the pathogenesis of Coronary Artery Disease (CAD). **(Albrink et al 1959**[3] **, Brown et al 1965**[11] **, Keys et al 1975**[52]**). Crowley and his Associates** in **1971**[22] confirmed serum lipid concentration in patients with CAD by coronary arteriograph. In the presence of hypercholesterolemia, the platelets become hyperactive which will increase the tendency toward platelet thrombus formation **(Carvalho et al 1974**[18]**, Stuart et al 1980**[91]**, Davi et al 1992**[26]**).** A report by **Cohn et al in 1976**[19] stated that CAD has continuous relationship with concentration of serum cholesterol and to lesser extent serum triglycerides.

Keys et al in **1980**[53] confirmed that hypercholesterolemia as a risk factor for IHD and coronary artery disease mortality. Risk of MI was directly related to concentration of cholesterol and LDL- C and inversely related to HDL-C **(Gordon 1981**[44]**)**

Although studies of lipid**s** and the degree of coronary atherosclerosis began in the late 1960s, uncertainty remained up to now over which lipid measurement discriminates the degree of CAD. Discussions over which is the "most influential" lipid parameter have been particularly unrewarding .Both TC and HDL cholesterol, when considered together, remained strongly related to the presence and extent of CAD**.** This denotes the importance of TC and HDL cholesterol as predictors of CAD**.**

Lowering blood cholesterol levels reduces coronary events in subjects both without (**Frick et al 1987**[36]**, Law et al 1994**[57]) and with (**Rossouw et al 1990**[82]**, Buchwald et al 1990**[14]**, Brown et al 1990**[13]) known coronary disease. Secondary intervention studies have demonstrated that decreasing LDL cholesterol and

increasing HDL-C in dyslipidemic patients with angiographically assessed CAD are accompanied by less progression and more regression of atherosclerotic lesions.(**Blankerhorn et al 1993**[10]). Improved endothelial function (**Drexler et al 1991**[28]) and inadequate plaque stabilization due to a decrease in the lipid content of the lesions most likely to rupture **(Brown et al 1993**[12]). These are two mechanisms that could partly account for the reduction in coronary events with cholesterol lowering. Another mechanism is a decrease in the tendency toward platelet thrombus formation with cholesterol lowering.

Ignasi Bolibar[48] and his associates on behalf of the Angina Pectoris Study Group in **1995** emphasized the role of serum lipid levels in determining not only the presence but also the extent of atherosclerotic disease in coronary arteries.

DIABETES AND CVD:

There are numerous plausible biological mechanisms to explain the early development of atherosclerosis and poor outcome of patients with diabetes and CVD. Patients with Type 2 diabetes develop abnormal endothelial function, platelet hyperactivity, aggressive atherosclerosis, a propensity for adverse arterial remodeling, enhanced cellular and matrix proliferation following arterial injury, and impaired fibrinolysis with a tendency for thrombosis and inflammation.

In an early case control study of 101 diabetic subjects, triglyceride was significantly associated with atherosclerosis in both men and women (**Santen et al 1972**[87]). It is well established that risk for CHD is increased in diabetics and that risk factors levels are also elevated.(**Garcia et al 1974**[40]**, Jarrett et al 1982**[47])

Four large prospective studies have shown that hyperinsulinemia is a predictor of coronary artery disease (CAD), with a few prospective reports not demonstrating

such a relationship. The greatest association of hyperinsulinemia with CAD has been found in Finland in a population with a very high frequency of CAD .Insulin resistance has been hypothesized to play a major role in dyslipidemia in individuals with normal glucose tolerance, as well as in those with impaired glucose tolerance and type 2 diabetes .(**Garg 1988**[41]**)**

Impaired endothelial function is not only antecedent to the development of atherosclerosis but also is demonstrable in the early stages of diabetes **(Mc.Veigh et al 1992**[67]). Several studies have shown a relationship between carotid wall atherosclerotic lesions, angina, and insulin levels/resistance **(Saloma et al 1995**[85]). Results of a prospective investigation of 2103 men from Quebec clearly showed that high fasting insulin concentrations are an independent predictor of CAD (**Desperes et al 1996**[27]). This important study used an insulin assay without cross-reactivity with proinsulin, thus avoiding that confounding influence.

` Abundant evidence shows that patients with type 1 diabetes or type 2 diabetes are at high risk for several cardiovascular disorders: coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure. Cardiovascular complications are now the leading causes of diabetes-related morbidity and mortality.

Prospective studies, such as the **Framingham**[40]**, Honolulu, and San Antonio Heart Studies**, as well as numerous more recent population studies in the United States and other countries, have documented the excess CVD risk in patients with diabetes from multiple racial and ethnic groups

The American Diabetes Association and the Juvenile Diabetes Foundation International have long emphasized the importance of identifying and applying interventions that help patients with diabetes reduce their risk for CVD. In addition,

the growing importance of diabetes as a cause of CVD has led the American Heart Association (AHA) to formally designate diabetes as a major risk factor for CVD. This will place diabetes on a coequal status with cigarette smoking, hypertension, and cholesterol disorders as major CVD risk factors.

Recent controlled trials of cholesterol-lowering therapy, particularly secondary prevention trials, showed that reducing low density lipoprotein cholesterol levels results in a striking decrease in major coronary events in patients with type 2 diabetes. **The Diabetes Control and Complication Trial (DCCT[46])** showed that the improved glycemic control can prevent or reduce microvascular disease and may reduce macrovascular disease in patients with type 1 diabetes.

The United Kingdom Prospective Diabetes Study (UKPDS[46]) demonstrated similar benefits in decreasing microvascular disease by controlling hyperglycemia in patients with type 2 diabetes and also reported that glycemic control probably reduces macrovascular disease. Recent studies suggest that the absolute risk for major coronary events in patients with type 2 diabetes approaches that of nondiabetic patients with established coronary heart disease. Worse, once patients with diabetes develop clinical coronary heart disease, they have a particularly bad prognosis, both acutely in the postinfarction period and over the long term.

It is important to recognize that the pathogenesis of diabetes-associated CVD is only partially understood and that expanded basic and clinical research is needed to determine the best and most efficacious ways to reduce cardiovascular complications in these high-risk patients**. (Grundy SM 1999[46])**

INFLAMMATION AND CVD:

Epidemiological and clinical studies have shown strong and consistent relationships between markers of inflammation and risk of future cardiovascular events. Inflammation occurs in the vasculature as a response to injury, lipid peroxidation, and perhaps infection.

Early in the process, in response to oxidized low-density–lipoprotein cholesterol (LDL-C), injury or infection, resident or circulating leukocytes bind monocytes to the site of a developing lesion. As they continue to ingest chemically modified lipids and lipoproteins, monocytes become macrophages, or foam cells, and initiate fatty streaks. In **1994, Moreno et al**[72] stated that more than half of all cells at the immediate site of plaque rupture are macrophages; they are the dominant type of atherosclerotic inflammatory cell infiltrates. At the same time, other inflammatory mediators, including activated T cells and mast cells, also attach themselves to the endothelium. All of these inflammatory cells eventually contribute to the formation of the atheromatous lesion which consists of a lipid pool protected by a fibrous cap. The monocyte-macrophages release metalloproteinases. These proteolytic enzymes can break down collagen in the fibrous cap, leaving it prone to rupture, and exposing the tissue factor and atherosclerotic debris beneath to arterial blood, inducing thrombosis.

Fuster et al in 1994[37] demonstrated that risk of plaque rupture correlates poorly with the degree of stenosis: half of all infarctions occur in arteries that have _50% luminal diameter narrowing. **Falk et al 1995**[31] evaluated the risk of plaque rupture exists with a thin fibrous cap, and inflammatory cells within and under the cap In a quarter of cases, plaque does not rupture; the endothelium is simply replaced by the prothrombotic inflammatory cells. These cells also release procoagulant tissue factor, which contributes to thrombogenesis .Smooth muscle cells (SMCs) secrete

factors that recruit additional monocytes. Local stimulation of SMCs in the artery wall can amplify the inflammatory response and promote a local procoagulant effect. (**Libby et al 2001**[58]).

The final steps in the inflammatory cascade occur when plaque contents— cholesterol, macrophages, tissue factor, necrotic debris, and platelet derived prothrombotic substances (thromboxane A2, serotonin, adenosine diphosphate, platelet-activating factor)— come into contact with the blood and thrombosis ensues. The results may be either coronary or cerebral infarction, depending on the duration of the thrombosis and the location of the associated vasoconstriction. (**Willerson 2002**[98]**).**

Thus inflammation plays a role in all stages of atherothrombosis, the underlying cause of approximately 80% of all sudden cardiac death (**Albert et al 2002**[2])

INFECTION AND INFLAMMATION INDUCE LDL OXIDATION IN VIVO

Some studies have suggested that specific infectious agents play a direct role in the vessel wall in the formation of atherosclerotic lesion,both infection and inflammation are accompanied by a systemic host response known as the acute-phase response (APR)(**Mendall MA, 1995**[69]).

Plasma ceruloplasmin levels are increased during Acute phase response, and purified ceruloplasmin has been shown to increase oxidation of LDL in cell-free systems as well as in cultured endothelial, smooth muscle (**Gitlin 1988** [42])

Research has recently shown that human atherosclerotic plaques contain massive amounts of lipid peroxidation products, despite the presence of large quantities of a-

tocopherol and ascorbate. (**Suarna C, 1995**[92]**).**APR is accompanied by alterations in lipid metabolism that include increased serum triglycerides and decreased HDL levels**. (Gabay 1999**[38])

Transferrin is another metal-binding protein associated with HDL. Hepatic synthesis and serum levels of transferrin are decreased during APR. APR may lead to less transferrin in HDL, which makes it less effective for protection of LDL against oxidation. (**Kunitake et al 1992**[56])

The LPS-induced increase in serum triglycerides occurs within 90 minutes, whereas changes in oxidized lipids are not seen until 24 hours after LPS treatment, which indicates that increase in oxidized lipids in serum is not simply a result of increased availability of fatty acid substrate. These results indicate that the host response to infection and inflammation is a potent stimulus for producing oxidation of serum lipids, including circulating LDL. (**Feingold et al 1992**[34])

Several structurally unrelated antioxidants slow the progression of atherosclerosis; whereas oxidized lipids in the diet enhance atherosclerosis (**Staprans et al 1996**[90].).Oxidative modification of lipoproteins plays a central role in the pathogenesis of atherosclerosis. Lipoproteins with oxidative damage and lipid peroxidation products have been detected in atherosclerotic lesions (**Berliner et al 1996**[8])

Circulating LDL is protected from oxidative stress by HDL associated enzymes, particularly paraoxonase, which destroys biologically active oxidized phospholipids (**Mackness et al 1996**[62]).

Van Lenten et al[95] have shown that serum paraoxonase activity is decreased in rabbits after croton oil administration and paraoxonase depleted HDL is unable to protect LDL from oxidation in vitro. **Feingold et al**[33] reported that LPS, Tumor

necrosis factor, and Interleukin-1 decrease serum paraoxonase activity and hepatic paraoxonase mRNA levels in Syrian hamsters in vivo which suggests that the decrease in paraoxonase is a feature of APR.

Increased LDL oxidation that occurs during infection and inflammation could be one of the mechanisms that promote atherosclerosis in patients with chronic infections and inflammatory diseases.

PERIODONTAL DISEASE AND CVD:

Korn et al in 1962[55] stated that transient bacteremia occurs with manipulation of periodontal tissues .Gingivectomy and root planning are reported to cause bacteremia in 55 to 80% of cases.

In the late 1970s, experimental infection of germ-free chickens with an avian herpesvirus induced an arterial disease that resembled human atherosclerosis (**Minick *et al.*, 1979**[70]). This finding initiated the 'systemic infection-heart disease' paradigm

The invasiveness of procedures as a cause of distant site infection is therefore unclear and little is known about the relative incidence, nature magnitude and duration of bacteremia from a wide variety of dental procedures.

(**Guntheroth,1984**[47]).

Clinicians have noted that periodontitis patients & ischemic heart disease patients have a number of characteristics in common. Studies have reported that heart disease is the condition most commonly found in periodontitis patients.(**Nevy et al 1987**[74]). **Mattila and colleagues** were the first to show a statistical association between dental infections and advanced coronary atherosclerosis (**Mattila *et al.*, 1989b**[66]).According to the study by Syrjänen and co-workers, dental infection was associated with cerebral infarction in young and middle-aged men (**Syrjänen et al.,**

1989[93]) and the study by **Paunio *et al.* (1993**[79]***)*** reported that missing teeth were associated with ischemic heart disease.

Destructive periodontal disease which involves Gram-negative bacteria, has been reported to be a significant predictor of coronary heart disease **(Beck *et al.*, 1996**[7]**)**. Because both coronary heart disease (CHD) and periodontal disease have a multi-factorial etiology, as well as a wide variety of possible confounding factors, a clear-cut consensus on the importance of the relationship between these two conditions has been difficult to obtain

Loesche *et al.*(1998[60]***)*** published a study in which the confounding variables were well-controlled, and the results seemed to suggest a positive association between dental disease and cerebrovascular accidents in US veterans. However, this study was cross-sectional and causal inferences could not be made.

Since cardiovascular diseases are the leading cause of death worldwide, greater attention has been focused on evidence that infections of the oral cavity might be associated with atherothrombosis: heart infarction, stroke, and peripheral vascular disease **(Slavkin and Baum, 2000**[89]**)**.

In Sweden, **Buhlin** and co-workers have published a national questionnaire study in which a significant association was observed between self-reported bleeding gums and CVD and between the presence of dentures and CVD. The data analysis was adjusted for age, smoking, income level, socio-economic and marital status, and education **(Buhlin *et al.*,2002**[15]).The same group also reported results from another questionnaire which suggested that the risk of CVD was increased if the patient had experienced problems with his/her teeth in the absence of dental care. Here, the odds ratio for the association of self-reported bleeding gums and CVD was 3.07 (CI 1.288–7.313) (**Buhlin *et al.*, 2003a**[16]**)**.

In the last ten years, several epidemiological studies have assessed the association between oral infection and systemic disease (**Renvert, 2003**[80]). These studies have provided support that oral infections, specifically periodontitis, may confer independent risks for different systemic conditions (mortality, osteoporosis, diabetes mellitus, pulmonary infections, pre-term low-weight births, cardiovascular diseases, and infections in other body sites).

Nevertheless, we agree with **Mattila (2003**[65]***)***, the original presenter of the hypothesis linking dental infection and CVD, that the measurement of alveolar bone loss as recommended by **Rutger Persson *et al.*(2003**[83]***)*** might be the best way to assess the severity of periodontal disease in large studies, because the radiographic assessment can be done in a blinded fashion, and several independent researchers can be used.

Richard Castillo in 2009[81] studied the relationship between atherosclerosis and tooth loss and found that atherosclerosis and dental loss are age dependent and are interrelated independent of age.

PATHOPHYSIOLOGY

Studies in humans/baboons have shown a number of cytokines are produced in response to systemic Gram-negative LPS exposure. Two principal cytokines involved in this response are TNF-alpha and IL- 1beta. Their appearance in the plasma has a well-defined temporal relationship indicative of a "cytokine cascade." TNF- alpha is the first factor in this cascade followed by IL-1 beta. It is believed that these cytokines exert effects on lipid metabolism by influencing production of other cytokines, altering hemodynamics/amino acid utilization of various tissues involved in lipid metabolism, or modifying the hypothalamic-pituitary-adrenal axis increasing plasma concentrations of adrenocorticotropic hormone, cortisol, adrenaline, noradrenaline,

and glucagon. Thus, through action of TNF- alpha and IL-1 beta, exposure to microorganisms/endotoxin results in elevated levels of free fatty acids (FFA), LDL, and TRG. These elevations in serum lipids are thought to arise from enhanced hepatic lipogenesis, increased adipose tissue lipolysis/blood flow, increased synthesis or reduced clearance of TRG, and reduced clearance of LDL due to reductions in lipoprotein lipase activity. Thus, any condition producing elevations in serum IL-1 beta /TNF- alpha has potential to cause hyperlipidemia. In the case of periodontitis, elevations of these cytokines may be mediated by "systemic dumping" of locally produced IL-1beta/TNF-alpha and/or low-level "asymptomatic bacteremia/endotoxemia." .This would be especially problematic in diabetic patients who may already exhibit elevated serum lipids and a systemic monocytic hyper-response trait.

PERIODONTITIS AND HYPERLIPIDEMIA:

Animal studies have demonstrated a causal relationship between hyperlipidemia and periodontitis. Investigations in rats by **Ueno et al in 1965**[94] have demonstrated that animals placed on high fat diet develop periodontitis.

The study of **Feingold et al 1992**[34] showed that the administration of low doses of endotoxins in rats resulted in hypertriglyceridemia suggesting the presence of a similar response in local infections such as periodontal disease.

The study of **Memon et al 1993**[68] has proved that induction of periodontitis by P.gingivalis in rats resulted in increased level of TGL. Using similar methodology, the same result was observed in work of **Doxey et al in 1998**[29].

Studies in non human primates by **Ebersole et al in 1999**[30] have demonstrated elevations in serum lipids of inflammatory biomarkers and LDL/TRG during

gingivitis and periodontitis. Additionally these changes were exacerbated by high fat diet and animals on high fat diet exhibited more severe periodontitis.

Human studies appear to confirm the hyperlipidemia periodontitis relationship. Subjects with hyperlipidemia demonstrated significantly more severe periodontitis than community based controls and the degree of periodontal breakdown was positively correlated to plasma lipid levels (**Pohl et al 1995**[78]). A study measuring serum lipid levels in systemically healthy patients with moderate periodontitis by **Netea et al in 1997**[73] showed a significant elevation of serum LDL/TRG in periodontitis patients compared to controls.

Studies involving clinical evaluation/ laboratory assessment of healthy and diabetic patients suggest a potential linkage between periodontitis, elevated serum lipid and level of bacteremia(**Cutler et al 1999**[23]) . Analysis of sera from random healthy clinic patients demonstrated significant elevations of serum lipids and P.gingivalis titers in patients with periodontitis compared to patients without periodontitis. It is also demonstrated significant association of the presence of disease with total serum cholesterol, TRG and amount of P.gingivalis antibodies (**Cutler et al 1999**[24]).

According to National Cholesterol Education Program periodontal disease was more prevalent in subjects with a serum HDL-C concentration <60mg/dl suggesting that periodontal disease is exacerbated by metabolic syndrome. (**NCEP 2000**).

Losche et al in 2000[59] showed that the plasmatic level of lipids in periodontitis patients were significantly higher than the healthy individuals.

Noack et al in 2000[75] studied 100 subjects and suggested that hyperlipidemia is a risk factor for periodontal disease, while impaired glucose tolerance is not.

In **2002, Katz et al**[51] evaluated the periodontal health of over 10000 Israeli military service men and women and compared the results with their blood lipid levels and did not find a significant association between the presence of periodontal pockets and high levels of triglycerides.

While some authors like **Katz et al 2001**[50]**, 2002**[51] mentioned that there were significant correlations between periodontal status and cholesterol levels, whereas **Cutler et al 1999**[24]**, Morita et al 2004**[71] **,** indicate that there were significant association between trigyceride level and periodontal disease.

A study by **Aki Izumi et al on 2009**[1] showed that higher total cholesterol is associated with lower prevalence of periodontitis in non smoking elderly patients.

After periodontal treatment, significant increase in serum HDL-C concentration, HDL2/HDL3 and HDL phospholipids content were observed. In this study, **Pussinen et al 2004**[77] stated that periodontitis decreases the antiatherogenic potency of HDL.

PERIODONTITIS AND DIABETES:

Williams and Mahan (1960[100]**)** reported a significant reduction in insulin requirements in 7 of 9 patients with diabetes and periodontal disease who underwent periodontal therapy. As such, diabetes may be adversely influenced by periodontal disease and conversely, periodontal disease may be more severe in the diabetic patient. This is important in that periodontal therapy may alter the patient's insulin requirement, thus requiring an adjustment in the insulin dosage. Therefore, it is important to inform the patient's physician prior to initiating periodontal therapy.

Glavind et al. (1968[43]**)** studied controlled diabetics aged 20 to 40 and compared them to a normal population. The investigators found no difference in the

populations up to the age of 30 years; however, patients older than 30 or patients who had been diagnosed with diabetes longer than 10 years had significantly more periodontal attachment loss.

Cohen et al. (1970[20]) compared diabetic and non-diabetic patients over a 2-year period and consistently observed more gingival inflammation and attachment loss in the diabetic patients at each examination. Increased glucose has been found in the gingival crevicular fluid (GCF). This altered the environment and might allow the growth of different subgingival bacteria and/or alter PMN function (**Kjellman et al., 1970**[54]). Polymorphonuclear leukocytes (PMNs) functions, such as chemotaxis and phagocytosis, have been shown to be decreased in diabetic patients with periodontal disease **(Manouchehr-Pour et al., 1981[63]).**

Cianciola et al. (1982[19]) demonstrated increased bone and attachment loss (periodontitis) in IDDM patients, compared to controls (siblings of the diabetics and non-related, nondiabetic patients) despite comparable plaque indices.

In contrast to the above studies**, Barnett et al. (1984**[6]) examined forty-five 10-to-18-year-old diabetic patients and found no correlation between the degree of diabetic control and the duration of diabetes compared to either the gingival index or the periodontal index. While this study conflicted with previous studies, it should be pointed out that none of the patients in this study exhibited any loss of interproximal bone, thus making comparison with previous studies difficult. In addition, these subjects were a rather homogeneous group from a private endocrinology practice and were probably very compliant.

Novaes et al. (1991[76]) compared the periodontal status of 30 Brazilian IDDM patients ages 5 to 18 with non-diabetic controls. They concluded that: 1) a statistically higher accumulation of plaque occurs among the diabetic patients (1.23 versus 0.81),

among female diabetics (1.34 versus 1.10), and among older patients (difference significant at the 5% level); 2) the gingival index was higher among diabetics than controls (0.52 versus 0.15), with no significant difference with respect to age and sex; 3) probing depth did not differ significantly between diabetics and controls in relation to increasing age, but in relation to sex, diabetic females showed deeper pockets in the palatal region; and 4) alveolar bone loss was significantly greater in diabetics than in non-diabetics in the upper and lower anterior region.

Seppala et al. (1993[88]) evaluated the progression of periodontal disease in subjects aged 35 to 55 years with longstanding, insulin-dependent diabetes mellitus. They report that under similar plaque conditions, poorly-controlled diabetics have more gingivitis, more bleeding on probing, greater loss of attachment, and more bone loss than well-controlled subjects.

Therefore considering the diversity of the few developed studies, as well as the different eating habits and studied populations, new researches to establish the real systemic changes caused by periodontitis are appropriate in order to explain the metabolic and physiologic changes responsible for changes in general health and in the increased susceptibility for certain systemic diseases.

MATERIALS AND METHOD

SUBJECT SELECTION

On getting approval from institutional ethical committee, from among the subjects who attended the Out patient clinic, Department of Periodontics, TamilNadu Government Dental College and Hospital, Chennai – 600 003, thirty individuals with Healthy Periodontium were taken as control group and thirty patients diagnosed with Chronic Periodontitis were taken as the Study Group.

INCLUSION CRITERIA

Control group

This included 30 individuals with the following features:

- Bleeding on Probing
- Absence of Clinical Attachment loss as determined by CAL (Clinical Attachment Level) measurements i.e. CAL=0.

Study Group

The patients in the study were divided into mild, moderate and severe based on CAL.

Mild - 1-2mm of CAL

Moderate - 3-4mm of CAL

Severe - >5mm of CAL

EXCLUSION CRITERIA

- Diabetes Mellitus
- Myocardial Infarction
- Stroke

- Dental treatment during past 6 months
- Smoking
- Patients on statins / any drugs against hypercholesterolaemia.
- Pregnancy/ Lactation
- Liver dysfunction
- Hypertension

STUDY PROTOCOL

1. Approval by the institutional ethical committee
2. Medical History and Informed Consent
3. Periodontal Examination using clinical parameters namely Probing Pocket Depth and Clinical Attachment Level.
4. Collection of blood samples
5. Enzymatic estimation of lipid profile and glucose levels

Following selection of subjects, written informed consent, which was approved by the Institute's Ethical Committee, was obtained from all the subjects selected for the study after explaining the study procedure. Examination was preceded by a thorough medical and dental history of the subjects. Intra-oral examination was done using mouth mirror and William's Periodontal Probe. Periodontal Evaluation was done by measuring the Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL).

CLINICAL PARAMETERS (17, 45)

PROBING POCKET DEPTH (PPD)

Probing Pocket Depth was measured from the gingival margin to the base of the pocket using William's Periodontal Probe. The probe was passed within the gingival sulcus along the circumference of the tooth.

Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, Distolingual).

$$\text{Probing depth per tooth} = \frac{\text{Sum of all scores per tooth}}{6}$$

$$\text{Mean probing depth per person} = \frac{\text{Sum of tooth score}}{\text{Total number of teeth examined}}$$

CLINICAL ATTACHMENT LEVEL (CAL)

Clinical Attachment Level was measured from the Cemento – Enamel Junction (CEJ) to the base of the pocket using William's Periodontal Probe.

- When the gingival margin was located on the anatomic crown, the level of the attachment was determined by subtracting from the probing pocket depth, the distance from the gingival margin to the CEJ. If both were the same, the loss of attachment was calculated to be zero.
- When the gingival margin coincided with the CEJ, the loss of attachment was calculated as equaling the probing pocket depth.

- When the gingival margin was located apical to the CEJ, the loss of attachment was greater than the probing pocket depth and therefore the distance between the CEJ and the gingival margin were added to the PPD.

Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

$$\text{CAL per tooth} = \frac{\text{Sum of all scores per tooth}}{6}$$

$$\text{Mean CAL per person} = \frac{\text{Sum of tooth score}}{\text{Total number of teeth examined}}$$

BLOOD SAMPLE COLLECTION

Sterile disposable 2ml syringe, with 23 gauge disposable needle, were used to obtain the required blood samples. Venous blood samples were obtained after 12 hours fasting, by venepuncture of cubital vein in the anticubital fossa. The blood was then transferred to previously sterile test tubes containing EDTA and transported to the clinical laboratory for processing.

CHOLESTEROL (ENZYMATIC COLORIMETRIC TEST)

PRINCIPLE

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterases hydrolyses the esters, and H2O2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol oxidase according to following reaction.

Cholesterol

Cholesterol esters + H_2O_2 ----------> Cholesterol and fatty acids.

Esterase

Cholesterol

Cholesterol + O_2 ----------> Cholesten-3-on + H_2O_2

Oxidase

Peroxidase

$2H_2O_2$ + Phenol + 4 aminoantipyrine ----------> quinoneimine dye + $4H_2O$

REAGENT CONCENTRATION

Reagent / R1 (Buffer)

Pipes buffer, PH 6.9	90mmol/litre
Phenol	26mmol/litre

Reagent / R2 (Enzyme Reagent)

Cholesterol oxidase	200 U/I
Cholesterol esterase	300 U/I
Peroxidase	1250 U/I
4-Aminoanipyrine	0.4mmol/l

Reagent / R_4 (standard)

Cholesterol	200 mg/dl (5.17 mmol/l)

PREPARATION AND STABILITY

Dissolve contents of enzyme reagent / R_2 with corresponding volume of buffer / R_1.

SAMPLES

Serum, Plasma collected with sodium citrate.

PROCEDURE

Wavelength	:	546nm (500-550nm)
Temperature	:	+25/ +30/+37^{O} C
Cuvette	:	1cm light pathway
Zero adjustment	:	Reagent blank one reagent blank per series only.

	Blank	**Standard**	**Sample**
Standard	-	10µl	-
Sample	-	-	10µl
Working Reagent	1000µl	1000µl	1000µl

Mix, measures after incubating at +37^{O} C for 5mins. Within 60 minutes read absorbance of ample against reagent blank.

CALCULATION

By standard

$$\frac{\Delta \text{ A sample}}{\Delta \text{ A sample}} \quad X \quad \text{standard conc.} = \text{Cholesterol concentration}$$

Normal value - < 200mg/dl

HDL CHOLESTEROL

PHOSPHOTUNGSTIC ACID METHOD – END POINT

METHODOLOGY

Burstein *et al*

PRINCIPLE

Chylomicrons, LDL, & VLDL (Low and Very low density lipoproteins) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using ERBA cholesterol reagent.

	Phosphotungstate			
Serum/Plasma	---------->	HDL	+	(LDL + VLDL)
	Mg^{2+}	(supernatant)		(Precipitate)

REAGEANT COMPOSITION

Reagent I: Precipitating Reagent

Phosphotungstic Acid	:	2.4mmol/L
Magnesium Chloride	:	40mmol/L

Reagent II: HDL cholesterol standard

HDL cholesterol standard : 25mg/dl

Sample:

Unhemolysed serum or plasma

PRECIPITATION

Precipitation of LDL

Pipette	Volumes
Sample	250µL
Precipitant Reagent	500 µL

Mix well and allow the reaction mixture to stand for 10 mins at room temperature, centrifuge at 400rpm for 10minutes to obtain a clear supernatant. Use the supernatant to determine the concentration of HDL cholesterol in the sample.

ASSAY PROCEDURE

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Cholesterol working Reagent	100µl	1000µl	1000µl
Distilled Water	50µl	-	-
HDL standard	-	50µl	-
Supernatant	-	-	50µl

Mix well, incubate for 10min at 37ºC or 12min at 30ºC read the absorbance of standard and each sample at 510nm or 510/630nm for bichromatic analyzer against reagent blank.

$$\text{HDL} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Value of standard (mg/dl)} \times \text{dilution factor}$$

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 25 \times 3$$

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 75$$

Normal value*:*

HDL > 40-50mg/dl

TRIGLYCERIDES

METHOD

Colorimetric Enzymatic test with turbidity cleanser.

PRINCIPLE

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol – 3 phosphate + ADP}$$

$$\text{Glycerol-3 phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy Acetone Phosphate + } H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine + 4 Chlorophenol} \longrightarrow \text{Dihydroxy + } 4H_2O_2$$

CONTENTS

R_1 - Mono Reagent.

R_2 - Standard 200mg/dl.

SAMPLE

Serum or Citrated Plasma

PROCEDURE

Pipette into cuvettes

	Blank	**Sample/standard**
Mono Reagent	1000µl	1000µl
Sample/standard	-	10µl

Mix, well incubate for 5 minutes at 37^{O} C or for 10 minutes at room temperature. Read the absorbance ΔA of standard/sample against blank.

CALCULATION

$$\text{Triglycerides (mg/dl)} = \frac{\Delta \text{ A Sample}}{\Delta \text{ A Sample}} \times 200$$

NORMAL VALUE:

Less than 150mg/dl.

LDL CHOLESTEROL

LDL Cholesterol is determined using Friedwald Formula

LDL-C = Total Cholesterol – HDL-C + Triglycerides / 5.

NORMAL VALUE

< 130 mg/dl

GLUCOSE

PHOTOMETRIC DETERMINATION OF GLUCOSE

PRINCIPLE

$$\text{Glucose} + O_2 + H_2O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O_2$$

REAGENTS USED

Reagent 1

Phosphate buffer PH 7.0	:	100mmol/l
Phenol	:	5mmol/l
4-aminoantipyrine	:	0.5mmol/l
Glucose oxidase	:	15KU/I
Peroxidase	:	1 KU/I

Reagent 2:

Glucose

SAMPLE

Serum, Sodium citrated plasma

PROCEDURE:

Reagent temperature	:	$+37^{O}$ C
Wavelength	:	500nm. Hg 546nm
Light path	:	1cms

Measuring Temperature : $+37^{O}$ C

	Sample	**Standard**	**Blank**
Sample	10µl	-	-
Standard	-	10µl	-
Reagent	1000µl	1000µl	1000µl

CALCULATION

Glucose (mg/dl) = As / Astd X Conc. of Standard.

NORMAL RANGE:

90-110mg/dl

STATISTICAL ANALYSIS

In a one-way ANOVA, the F statistic tests whether the treatment effects are all equal, i.e. that there are no differences among the means of the J groups. A significant F value indicates that there are differences in the means, but it does not tell you where those differences are, e.g. group 1's mean might be different than group 2's mean but not different from group 3's mean.

To isolate where the differences are, you could do a series of pairwise T-tests. The problem with this is that the significance levels can be misleading. For example, if you have 7 groups, there will be 21 pairwise comparisons of means; if using the .05 level of significance, you would expect at least one statistically significant difference even if no differences exist.

Therefore, various methods have been developed for doing multiple comparisons of group means. In SPSS, one way to accomplish this is via the use of the /POSTHOC parameter on the One-way command. We'll present the SPSS output and then explain what the different parts mean.

MEAN DIFFERENCE.

This column gives the difference in the means of the 2 groups

STANDARD ERROR.

In a One-way Anova, the standard error of the difference between the two means of groups i and j is

$$s_{\bar{\mu}_i - \bar{\mu}_j} = \sqrt{MSE * \left(\frac{1}{N_i} + \frac{1}{N_j}\right)}$$

$$s_{\hat{\mu}_i - \hat{\mu}_j} = \sqrt{MSE * \left(\frac{1}{N_i} + \frac{1}{N_j}\right)} = \sqrt{2.6 * \left(\frac{1}{5} + \frac{1}{5}\right)} = \sqrt{1.04} = 1.0198$$

SIGNIFICANCE:

This column gives you the significance of the difference under the multiple comparison method being used. To understand this, we need explain following method being used and what their rationale is.

When the decision from the One-Way Analysis of Variance is to reject the null hypothesis, it means that at least one of the means isn't the same as the other means. What we need is a way to figure out where the differences lie, not just that there is a difference.

This is where the Tukey tests come into play. They will help us analyze pairs of means to see if there is a difference much like the difference of two means covered earlier

The formulas refer to mean i and mean j. The values of i and j vary, and the total number of tests will be equal to a combination of k objects, 2 at a time C(k,2), where k is the number of samples.

TUKEY'S HSD POST-HOC TEST

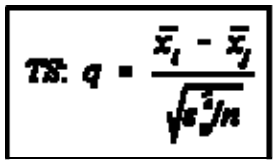
` A post-hoc test is needed after we complete an ANOVA in order to determine which groups differ from each other. Do not conduct a post-hoc test unless you found an effect (rejected the null) in the ANOVA problem. If you fail to reject the null, then there are no differences to find..

For the Tukey's post-hoc test we will first find the differences between the means of all of our groups. We will compare this difference score to a critical value to see if the difference is significant. The critical value in this case is the HSD (honestly significant difference) and it must be computed. It is the point when a mean difference becomes honestly significantly different.

$$HSD = q\sqrt{\frac{MS_{Within}}{n}}$$

Note that "q" is a table value, and n is the number of values we are dealing with in each group (not total n). The Mean Square value is from the ANOVA you already computed. To find "q" or the student zed range statistic, refer to your table on page A-32 of your text. On the table 'k' or the number of groups is found along the top, and degrees of freedom within is down the side. Cross index the row and column to find the value you need to put in the formula above.

The test statistic is found by dividing the difference between the means by the square root of the ratio of the within group variation and the sample size



INDEPENDENT t TEST

The independent t-test, as we have already mentioned is used when we wish to compare the statistical significance of a possible difference between the means of two groups on some independent variable and the two groups are **independent** of one another.

The formula for the independent t-test is

$$t = \frac{X_1 - X_2}{\sqrt{\left(\frac{SS_1 + SS_2}{n_1 + n_2 - 2}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}},$$

where

X_1 is the mean for group 1,

X_2 is the mean for group 2,

SS_1 is the **sum of squares** for group 1,

SS_2 is the **sum of squares** for group 2,

$\mathbf{n_1}$ is the number of subjects in group 1, and

$\mathbf{n_2}$ is the number of subjects in group 2.

The sum of squares is a new way of looking at variance. It gives us an indication of how spread out the scores in a sample are. The t-value we are finding is the difference between the two means divided by their sum of squares and taking the degrees of freedom into consideration.

$$SS_1 = \sum X_1^2 - \frac{\left(\sum X_1\right)^2}{n_1}$$

and

$$SS_2 = \sum X_2^2 - \frac{\left(\sum X_2\right)^2}{n_2}$$

We can see that each sum of squares is the sum of the squared scores in the sample minus the sum of the scores quantity squared divided by the size of the sample (n).

So to calculate the independent-t value we need to know:

1. The mean for sample or group 1
2. The mean for sample or group 2
3. The summation X and summation X squared for group 1
4. The summation X and summation X squared for group 2
5. The sample size for group 1 (n_1)
6. The sample size for group 2 (n_2)

We also need to know the degrees of freedom for the independent t-test which is:

$$df = n_1 + n_2 - 2$$

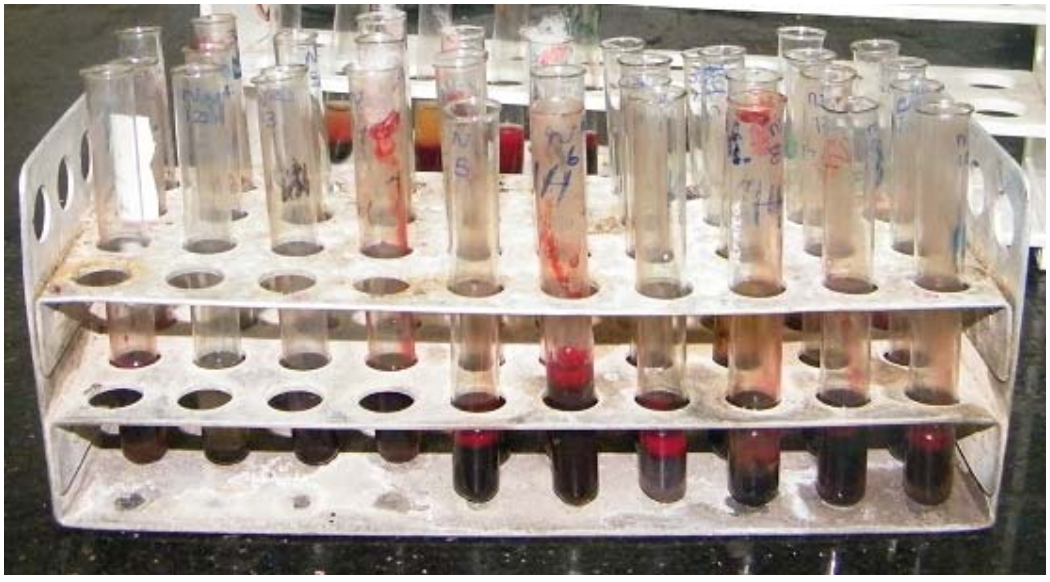
Photograph 1
Armamentarium

Photograph 2
Control Group (Healthy)

Photograph 3
Study Group (Chronic Periodontitis)

Photograph 4 Williams's periodontal probe used to measure probing pocket depth and CAL

Photograph 5
Collection of blood sample



Photograph 6
Collected blood sample

Photograph 7

Erba Chem 7 analyser used for estimating blood glucose and lipid levels

RESULTS

Sixty subjects were included in the study. Among them thirty subjects with CAL = 0mm were taken as controls (healthy periodontium) and thirty subjects divided into mild(CAL=1-2mm), moderate(CAL=2-3mm) and severe(CAL ≥5mm) were taken as study group.The clinical parameter used for analysis and comparison between the two groups was clinical attachment level (CAL) which is a measure of loss of attachment. Blood parameters used are glucose and lipid profile levels. The difference between the groups in the blood parameters were taken into consideration for analyzing whether periodontitis is a risk factor for CVD.

Table 1 and **Table 2** show the values of Clinical Attachment Level (in mm) and levels of blood glucose, total cholesterol, HDL, triglycerides, LDL, (in mg/dl) in Study and control groups respectively

Table 3 (Fig 1) shows the mean values and standard deviation of blood glucose and lipid levels in control and mild, moderate and severe periodontitis patients.

Mean values of glucose in severe periodontitis patients (103) is greater than that of controls (75.60) but the P value is not statistically significant. All the values of lipid parameters except LDL showed high mean values in study group compared to control group but the P value is not significant except in TGL. In all the parameters except LDL and HDL the mean values of severe periodontitis group is greater than that of control group as well as mild, moderate and severe periodontitis group.

In TGL, mean values of control is 86.60 where as in severe periodontitis it is 230.70. This is statistically significant with P value of 0.000.

Table 4 shows the proportion of abnormality in study group. This table shows that in glucose, the number of patients beyond the cut off value is 7 and among these 7, 4 of them belong to severe periodontitis group (57.14%).

In total cholesterol, the total number of patients beyond the cut off value is 6, among these 3 of them belong to severe periodontitis group. (50%).

In TGL, 11 patients out of 30 patients were above the cut off value and of these 10 patients belong to the severe periodontitis group (90.9%).

In HDL, the number of patients below the cut off value is 4, 2 (50%) belong to moderate periodontitis group and one of them belong to severe and another one belongs to mild periodontitis patients.

In LDL, the number of patient above the cut off value is 4 and out of 4, 3 (75%) of them belong to mild periodontitis group.

In all these parameters, except HDL and LDL, the maximum number of patients belongs to severe periodontitis group.

Table 5 shows the proportion of abnormality in control group. In this table, it shows the total number of patients beyond the pathological value is 3 out of 30 individuals. In the lipid parameter, all patients exhibited below the cut off value except HDL in which 8 of them were with pathological cut off value.

Table 6 shows the mean standard deviation and standard error mean values between study and control group.

In this table, independent t test was used and the values are matched between control and study group. Mild, moderate, and severe periodontitis patients in the study group were clumped into single group and analyses were made.

According to this test, mean values of all the blood parameters in the study group was greater than that of control except LDL in which the mean values of study group is 94.420 whereas in control group it is 96.327.

Table 7 shows the P values of the blood parameters using independent t test between control and study group.

In this, P value of total cholesterol is 0.002 and TGL is 0.000 which is statistically significant. Other values are not statistically significant.

Figure 2 shows the comparison of CAL in control and mild, moderate and severe periodontitis patients

Table1 master chart study group

Mild								Moderate							Severe						
S. no	Age/Sex	Glucose(mg /dl)	Total cholesterol	HDL	TGL	LDL	CAL	Age/Sex	Glucose(mg /dl)	Total cholesterol	HDL	TGL	LDL	CAL	Age/Sex	Glucose(mg /dl)	Total cholesterol	HDL	TGL	LDL	CAL
1	42/F	60	164	54	104	89.2	1.2	44/M	70	190	48	140	114	3.6	37/M	<60	146	43	238	55.4	5.3
2	42/M	73	126	42	102	63.6	1.2	52/M	56	153	48	142	76.6	3.8	70/F	109	208	42	182	129.6	5.08
3	35/M	<60	152	47	106	83.8	1.18	33/F	55	153	38	148	85.4	3.5	37/M	91	158	40	162	85.6	.5.02
4	40/F	<60	135	38	102	76.6	1.4	37/F	60	140	43	156	65.8	3.7	60/M	126	223	43	179	144.2	5,12
5	37/M	203	164	48	110	92	1.4	40/F	60	182	48	126	108.2	3.2	35/F	176	197	59	192	99.6	5.1
6	35/F	60	198	45	110	131	1.5	49/M	<60	192	51	118	117.4	3.1	45/F	74	213	45	291	109.8	5.2
7	45/F	60	125	35	120	66	1.8	36/M	90	141	39	122	77.6	3.2	46/F	61	186	42	224	99.2	5.2
8	33/F	60	200	41	105	138	1.3	40/F	126	207	45	120	138	3.2	35/F	99	148	42	239	58.2	5.2
9	40/M	60	227	43	108	162.4	1.2	37/M	75	147	47	128	74.4	3.3	50/F	76	162	40	276	66.8	5.2
10	63/M	112	178	`43	115	112	1.7	35/F	62	142	47	125	70	3.3	39/F	168	146	39	324	42.2	5.4

Table 2 Master chart (control group)

S. no	Age/Sex	Glucose(mg/dl)	Total cholesterol	HDL	TGL	LDL	CAL
1	50/F	<60	152	49	97	83.6	0
2	42/M	70	198	45	70	1284	0
3	36/F	115	150	45	105	84	0
4	40/M	70	141	47	104	73.2	0
5	60/F	89	168	42	106	104.8	0
6	35/F	70	186	43	72	128.6	0
7	42/M	84	162	45	98	97.4	0
8	45/F	60	148	40	98	92.4	0
9	40/M	60	174	40	96	114.8	0
10	40/F	<60	168	45	97	104	0
11	60/M	58	163	40	93	106.4	0
12	35/M	67	148	52	98	76.4	0
13	45/F	60	130	40	77	74.6	0
14	35/F	68	141	40	82	86.6	0
15	40/M	<60	125	40	61	78.8	0
16	42/F	<60	181	40	74	129.2	0
17	40/F	60	190	41	62	186.6	0
18	35/M	216	142	40	80	87	0
19	35/M	68	147	48	62	86.6	0
20	37/M	<60	152	40	57	104.6	0
21	49/M	157	149	40	84	93.2	0
22	36/M	79	146	42	98	84.4	0
23	41/M	84	153	42	90	93	0
24	42/F	70	149	42	70	93	0
25	36/F	70	154	42	87	94.6	0
26	40/F	60	152	49	97	83.6	0
27	42/F	60	132	42	96	70.8	0
28	37/M	70	140	45	107	73.6	0
29	52/M	83	152	47	82	88.6	0
30	47/F	70	162	40	98	104.4	0

***Table 3*: Blood lipid and glucose levels (mg/dl) mean and standard deviation in controls and mild, moderate and severe periodontitis patients(Study group)**

Blood parameters (mg/dl)	Control		Study						p-value
			Mild		Moderate		Severe		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Glucose	75.60	34.106	78.80	47.126	70.40	22.717	103.00	42.716	.178
Total cholesterol	156.00	19.946	166.90	34.129	164.70	25.272	178.70	30.137	.106
TGL	86.60	14.956	108.20	5.789	132.50	13.058	230.70	53.564	.000
HDL	42.37	4.319	43.67	5.657	45.40	4.195	43.50	5.720	.377
LDL	96.327	20.9494	101.460	33.154	92.74	24.654	89.060	33.527	.737

***Table 4*: Frequency of pathological values of plasma lipids and blood glucose levels in periodontitis patients**. (**Study group**)

Cut off (mg/dl)		Study	
		Under	Beyond
Glucose	>100	23	7
Total cholesterol	>200	24	6
TGL	>150	19	11
HDL	<40	4	26
LDL	>130	26	4

***Table 5*: Frequency of pathological values of plasma lipids and blood glucose levels in controls**

Cut off (mg/dl)		Controls	
		Under	Beyond
Glucose	>100	27	3
Total cholesterol	>200	30	0
TGL	>150	30	0
HDL	<40	0	30
LDL	>130	30	0

Table 6: **Mean and Standard Deviation of the Blood Glucose and Lipid levels in control and study group**

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Glucose(mg/dl)	Study Group	30	84.07	40.165	7.333
	Control Group	30	75.60	34.106	6.227
Total cholesterol	Study Group	30	170.10	29.676	5.418
	Control Group	30	156.00	19.946	3.642
HDL	Study Group	29	44.21	5.109	.949
	Control Group	30	42.37	4.319	.789
TGL	Study Group	30	157.13	62.088	11.336
	Control Group	30	86.60	14.956	2.731
LDL	Study Group	30	94.420	30.1097	5.4973
	Control Group	30	96.327	20.9494	3.8248
CAL	Study Group	30	3.32	1.588	.290
	Control Group	30	.00	.000	.000

Table 7**: p-values calculated used independent t test between control and study group**

Blood parameters	p-value
Glucose	.129
Total cholesterol	.002
TGL	.000
HDL	.493
LDL	.016

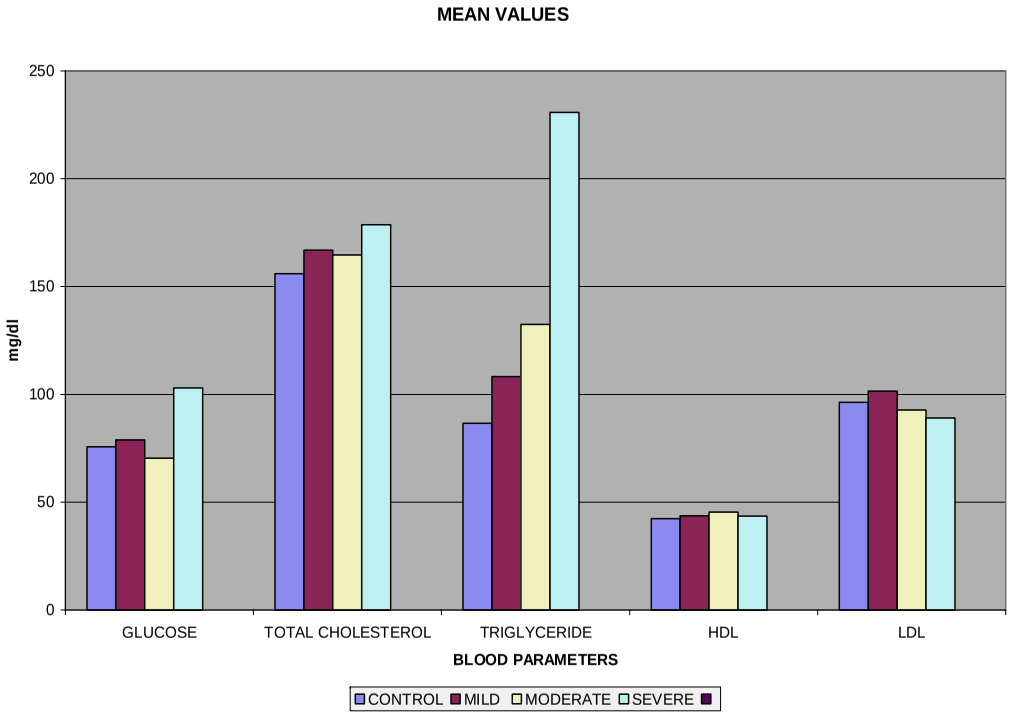


Figure 1 Mean values of blood glucose and lipid levels in control group and mild, moderate, severe periodontitis patients (Study group)

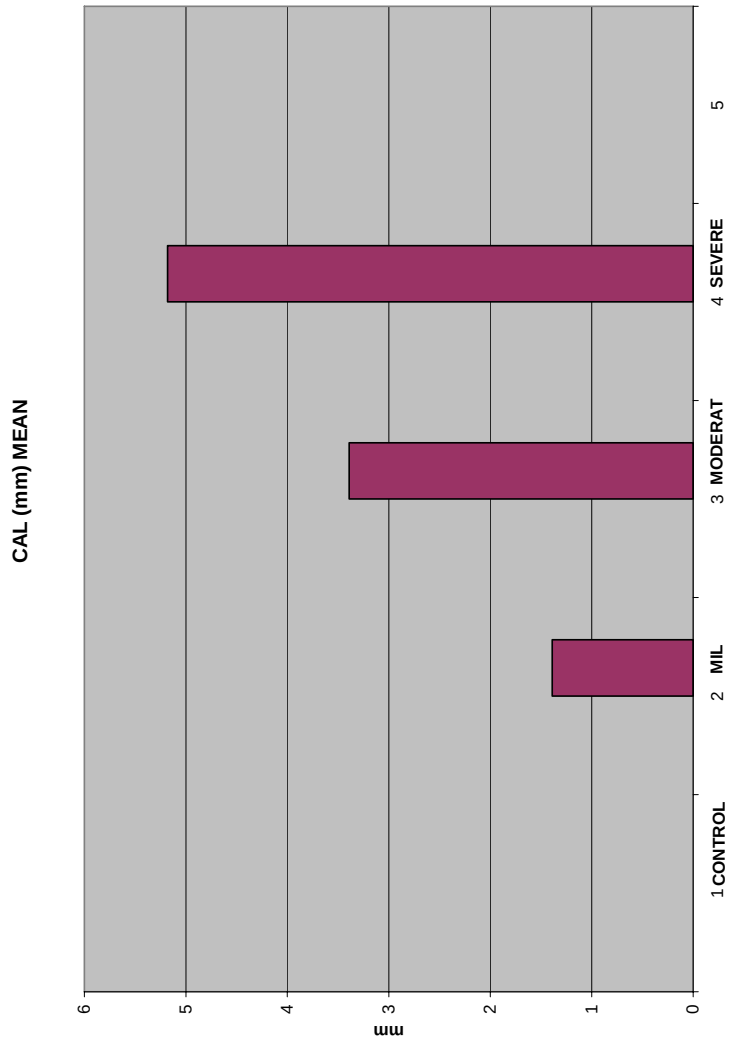


Figure 2 Comparison of CAL in control and mild, moderate, and severe periodontitis patients

DISCUSSION

The periodontium has been described as a potential reservoir of endotoxin, cytokines and lipid mediators in the body. The administration of low dosages of endotoxin in rodents rapidly induces changes in lipid metabolism resulting in hyper triglyceridemia. Infection with P.gingivalis or other gram negative periodontal pathogens could trigger the systemic release of IL-1 beta and TNF-alpha altering fat metabolism resulting in chronic hyper triglyceridemia. When periodontitis was induced in rats by administration P.gingivalis serum TGL increased (**Memon et al 1993**[68]).

In the present study both sexes were included similar to other studies (**Cutler 1999**[23] **Joseph Katz 2002**[51]**, Katz 2001**[50]**, Loesche 2000**[59]

Cutler et al[23] in his study selected sample with age group of 25 to 60 years but in the present study the age group selected is 35 to 70 years for both group.

The other confounding factors related to elevated cholesterol level like smoking, hypertension, lever disease were eliminated in the present study similar to earlier studies. (**Loesche 2000**[59]).

According to **Katz et al 2002**[51] smoking status is positively correlated with TC, LDL and TGL and negatively associated with HDL cholesterol. Vasoconstriction of gingival blood vessels caused by smoking promoted invasion of periodontal disease by micro organism (**Loesche 1998**[61]), Hence smokers was excluded from the study.

By following the earlier studies (**Christopher Cutler, 1999**[23]) patients with normal blood pressure were taken in the present study.

A hyper responsive monocytic state of chronic periodontitis results in further elevation of serum pro inflammatory cytokines and lipids hence patients with chronic periodontitis was taken in the present study (**Salvi et al 1998**[86])

In this study, CPITN probe was not used because it has been claimed that CPITN findings over estimated both prevalence and severity of periodontal attachment loss among young age groups, but underestimated these parameters among older subjects (**Baeleum et al 1995**[5]),instead Williams periodontal probe was used in this study.

Although there are several studies reporting the association between periodontitis and systemic lipid levels, the results remain controversial. In some studies (**Katz 2001, 2002**[50, 51]), TC and periodontitis exhibited significant relationship whereas in other studies (**Cutler et al 1999**[23]**, Morita et al 2004**[71]), TGL showed significant relationship to periodontitis. This discrepancy may arise from the methodological difficulties associated with the complexity of lipid metabolism and variety of metabolic lipid parameter.

In this study, the patients in the study group were categorized into mild, moderate & severe based on the CAL and compared with the controls for the blood lipids and glucose levels to investigate whether the lipid profile and glucose parameters alters with increasing severity of the study. Within the limits of our knowledge, no study has been done before correlating the severity of the disease with the lipid profile and glucose levels.

According to our results, plasma level of TGL was significantly higher in mild, moderate, and severe periodontitis group when compared with age & sex matched controls. (P>0.000).

This result was found to be consistent with **Matilla et al**[66] who found significant association between TGL and dental infection.

When mild, moderate and severe periodontitis were taken as one group & compared with control group, TC was statistically significant. This result was found to be concurrent with **Cutler et al**[23] who found that presence of periodontitis was significantly associated with increase in TC level but in this study TGL was not significantly associated with periodontitis.

In our study, the frequency of pathological TGL and TC levels were more prevalent in patients with periodontitis than in control in agreement with **Loesche et al**[59].However in this study, we did not find significant differences between the groups for LDL and HDL cholesterol.

Katz et al[51] suggested that patients with hypercholesterolemia have more severe periodontal disease. However in the same study, no association was found with regard to TGL**. Saito et al**[84] **and Wakai et al**[97] also did not mention a significant association between periodontal status and TGL level. **Morita et al**[71] indicated that elevated TGL level might be potential indicator for the presence of the periodontal disease. **Cutler et al**[23] reported that elevated TGL levels are able to modulate IL-1beta production by PMN that are stimulated by P.gingivalis LPS.

Anna Cristina et al 2005[4], found no significant relationship between periodontal disease, regardless of its intensity and blood lipid levels in the studied population.

Our findings indicate strong association between plasma TGL levels and periodontal disease. However some methodological limitation should be kept in mind when interpreting our result. First, because this study had a case control design, it is difficult to make causal inferences based on its findings. Second, the sample size is small to give a consistent decision. Thirdly, no intervention studies have been done.

Though diabetes is an exclusion criteria in our study, fasting glucose levels were measured. The level of glucose in severe periodontitis patients was not statistically significant when compared to controls, but the means of glucose levels in severe periodontitis was higher than that of controls.

The results of this study regarding blood glucose levels were in accordance with **Loesche et al 2000**[59]. This indicates that systemically healthy patients with severe periodontitis demonstrated significantly higher blood glucose levels than patients without periodontitis. This observation may indicate that patients with periodontitis have impaired glycemic control and are in a "prediabetic state."

Thus infection-induced insulin resistance syndromes, if longstanding or chronic, are considered to be precursors to active diabetes due to the pancreatic β-cell destruction that results from sustained elevations of IL-1beta/TNF-alpha. In fact, some investigators suggest that a "proinflammatory imbalance" created by IL-1beta/TNF-alpha is one of the most critical determinants of β-cell loss in diabetic patients (**Netea et al 1997**[73]).

It has been suggested that proinflammatory cytokines such as IL-1beta and TNF-alpha, produced as a systemic response to periodontal infection are responsible for insulin resistance and subsequent poor glycemic control in periodontitis patients. In order to determine whether periodontitis induces insulin resistance and a

prediabetic condition, future studies will need to evaluate prediabetes in a very precise and consistent manner.

The two way relationship between diabetes mellitus and periodontal disease has been studied intensively in the past and has been subjected to large controversies. Very few studies however have investigated the role of glucose level in periodontitis patient & vice versa in non- diabetic patients. (**Yousuf Khader 2008**[96]).

A relationship between infection and lipid and glucose levels has been widely documented in past decades. Chronic infectious diseases are now thought to have an impact on plasma lipid (**Gallin et al 1969**[39]). This dyslipidemia is thought to be a part of host response aimed at decreasing the toxicity of harmful microbiologic agent.

This study gives another evidence for an association between severe periodontitis and alteration of lipid levels, which may be mediated by constant activation of inflammatory process determined by the presence of periodontal pathogens in periodontal pockets. (**D' Auito et al 2005**[25]).

Bacteria such as P.gingivalis, one of the most common pathogens present in severe periodontitis are capable of stimulating a continuous release of pro inflammatory cytokines such as IL-1 IL-6, TNF alpha. These cytokines are believed to have direct or indirect effects resulting in enhanced elimination of TGL and increased lipolysis.(**Feingold & Grunfeld 1987**[32]).

The physical and chemical properties of cell membranes are largely determined by the nature of the fatty acids such as gamma linoleic acid within the phopholipid bilayer. Thus alteration of the lipids in the blood stream or tissues can have effect on molecules signaling via receptors. Lipids may interact directly with myeloid cells altering gene expression for pro inflammatory cytokines and essential growth factors. It is possible that abnormalities in macrophage function, caused by

elevations in serum lipids, alter the cytokine secretion necessary for normal wound healing to occur. Thus it is conceivable that periodontitis would be potential risk factor for CAD through long term alteration in fatty acid metabolism and potentiation of pro inflammatory cytokine production.

According to **American Journal of Cardiology & Journal of Periodontology, 2009**, patients with periodontitis meeting criteria for metabolic syndrome should be identified and all the risk factors for atherosclerotic CVD should be treated, beginning with lifestyle changes aimed at weight reduction.

Within the limits of case control design, this study supports the association between periodontitis and dyslipidemia which may predispose to more serious systemic complication.

Finally, although longitudinal evaluation with a larger population may be an important step in revealing the causal relationship between periodontal disease and impaired lipid metabolism, our study may shed light on the clarification of this association in the future.

SUMMARY AND CONCLUSION

Levels of blood glucose and lipid levels were determined in individuals of control group with CAL = 0 mm (healthy periodontium, n = 30) and in patients of study group with CAL ≥ 1-2mm (mild), CAL >3-4mm (Moderate), CAL>5mm(Severe)(Chronic Periodontitis, n = 30) by analyzing their blood samples from cubital vein in anticubital fossa. The blood was then transferred to previously autoclaved test tubes containing EDTA and transported to the clinical laboratory for processing. The data collected was statistically analyzed.

According to the present study,

- There is a statistically significant increase in the levels of TGL in the patients with Chronic Periodontitis when compared with those not affected by Periodontitis.
- When mild, moderate and severe periodontitis were taken as one group and compared with control group, TC was stastiscally significant.
- There is no significant difference in the levels of LDL-C, HDL-C and glucose between the patients of two groups.
- As the severity of the disease increases, alteration in the blood parameters also increases.
- Although the levels of blood glucose are elevated in patients with severe Chronic Periodontitis, this increase is not stastiscally significant. This may be due to the smaller sample size.

It is likely that severity and poor control of periodontal disease might affect the level of lipids and glucose in blood which may increase the risk for CVD. Therefore, when periodontitis is newly diagnosed in patients with atherosclerotic CVD, periodontists and physicians managing patients with CVD should closely collaborate to optimize CVD risk reduction and periodontal care in accordance with American Journal of Cardiology and Journal of Periodontology joint recommendation.

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APPENDIX . Proforma and Informed Consent

PROFORMA

Blood Glucose levels and Dyslipidemia in Chronic Periodontitis Patients:

Case Control study

Date: **Dental O.P. No:** **Code No.:**

Name : Age/Sex :

Address : Tel. no. : Mobile no. :

Occupation : Income :

Chief Complaints:

Pain / shaky teeth / Bleeding gums / Swollen Gums / Receding Gums /Increase in Spacing between teeth / Pus Discharge / Stains/others

Duration:

Medical History:

1. Diabetes mellitus
2. Pregnancy / Lactation
3. Cardiac diseases
4. Stroke
5. Any drugs against hypercholesterolemia
6. Liver dysfunction
7. Hypertension

Dental History

Periodontal treatment within past 6 months

Habits

Smoking

Code No:

Clinical Examination

PROBING DEPTH (PD**) AND CLINICAL ATTACHMENT LEVEL (**CAL**) (in mm)**

Maxillary:

Palatal

CAL																
PPD																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
PPD																
CAL																

Buccal

Mandibular:

Lingual

CAL																
PPD																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
PPD																
CAL																

Buccal

Control Group ☐ Study Group ☐

Code No:

Blood Sample Details:

Date:

BLOOD PARAMETERS

GLUCOSE-
LDL-
HDL-
TRIGLYCERIDES-
TOTAL CHOLESTROL-

INFERENCE

Signature of PG student

Date:
Time:

Informed Consent Form – English

Study Title

Blood glucose levels and Dyslipidemia in Chronic Periodontitis Patients; A Case control study

Name: **O.P. No:**

Address: **Code No.:**

Age / Sex:

I, _____Age_____yrs, exercising my free power of choice, hereby give my consent to be included as a participant in the study '**Blood glucose levels and Dyslipidemia in Chronic Periodontitis Patients; A Case control study"**.

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I understand that the lab investigations will require the procurement of my blood in required amount.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and Institution will keep my identity confidential.

Name of Patient Signature / Thumb impression
Date

Name of Investigator Signature
Date

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு:

நாள்பட்ட பல்லைச் சுற்றிய திசுவில் ஏற்படும் அழற்சி உள்ள நோயாளிகளின் ரத்தத்தில் உள்ள சர்க்கரை மற்றும் கொழுப்பின் மாற்றத்தின் அளவு பற்றிய ஓர் ஆராய்ச்சி.

பெயர் : .....புறநோயாளியின் எண்: .....

முகவரி: .....ஆராய்ச்சி சேர்க்கை எண்: .....

..... வயது :

..... பாலினம் ஆண் ☐ ☐ பெண்

நாள் .....வயது ..... என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன், இந்த மருத்துவ ஆராய்ச்சி என்னை கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களுக்கு நான் எனது சம்மதத்தைத் தருகிறேன்.

- இந்த ஆராய்ச்சியின் நோக்கம், மருத்துவ முறைகள் பரிசோதனை முறைகள் எனக்கு திருப்தி தரும் வகையில் விளக்கப்பட்டன.
- பரிசோதனை செய்வதற்காக என் உடம்பிலிருந்து இரத்தம் எடுக்கப்பட வேண்டியுள்ளதாக அறிகிறேன்.
- நான் எடுத்துவருகின்ற மற்றும் முன் உட்கொண்ட மருந்துகள் பற்றிய விவரங்களை ஆராய்ச்சியாளர்களிடம் அறிவிக்க சம்மதம்.
- என் உடல் நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத, வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்துவரிடம் தெரிவிப்பேன் என உறுதியளிக்கிறேன்.
- எனக்கும் மற்றும் மருந்து ஆராய்ச்சியாளருக்கும் இந்த ஆராய்ச்சியிலிருந்து எந்த ஒரு நிலையிலும் விலகுவதோ அல்லது விலக்குவதற்கோ முழு உரிமை இருப்பதாக அறிகிறேன்.
- என்னுடைய மருத்துவக் குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக் கொள்ள சம்மதிக்கிறேன். ஆராய்ச்சி மையமும், ஆராய்ச்சியாளரும் என்னுடைய பெயர் மற்றும் சில விவரங்களை இரகசியமாக வைப்பதாக அறிகிறேன்.

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நோயாளியின் பெயர்: கையெழுத்து தேதி:

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ஆராய்ச்சியாளரின் பெயர்:
கையெழுத்து தேதி: